FastLyse pDNA Miniprep Kit

User Guide

Membrane Technologies

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1. Introduction

The **mdi** FastLyse Miniprep Kit is the fastest and simplest method available to obtain high yield, high quality pDNA from E.coli. The kit incorporates a uniquely formulated buffer L to lyse the bacterial culture and fast spin column technology to purify it. This technology does away with cumbersome pellet formation and resuspension steps along with phenol extraction (associated with desalting) and ethanol precipitation (associated with anion exchange based purification).

2. Applications

1. Bacterial Transformation
2. Restriction Digestion
3. Ligation
4. Transcription
5. PCR
6. Sequencing

3. Storage Conditions

Optimum storage conditions at which all components of the kit can be preserved without reduction in it's quality and performance.

One Year	RT
Longer Storage	2-8°C

Important

1. After adding RNase A to buffer N, store at 2-8°C
2. In case of any precipitation is observed in buffer L, re-dissolve it by incubation at 30-37°C for 30 minutes and mix by inversion

4. Quality Assurance

The **mdi** FastLyse pDNA Miniprep Kit is designed for various pre determined specifications and user requirements such as yield, purity, ruggedness, shelf life and functional convenience.

These are produced through a well defined quality management system certified by Underwriters Laboratories, USA for ISO 9001 which ensures intra lot as well as lot to lot consistency.

5. Safety Information

The buffers and the reagents may contain irritants, so wear lab coat, disposable gloves and protective goggles while working with the FastLyse Miniprep pDNA Kit.

6. Lot Release Criteria

Each lot of FastLyse pDNA Miniprep Kit is tested against predetermined specifications to ensure consistent product quality.

7. Technical Support

At **mdi**, customers are our priority. We will share our experiences to assist you to overcome problems in general product usage as well as customize products for special applications. We will

* Stimulate problems, and suggest alternative methods to solve them.

* Make changes/ improvements in our existing products/protocols.

* Develop special new products and system especially to satisfy your needs.

We welcome your feedback to improve our products.

8. Kit Contents

Contents	Quantity			Storage Temperature
Spin Columns	50	250	1000	RT
Collection Tubes	50	250	1000	RT
Buffer TE	32ml	160ml	640ml	RT
Buffer L	10ml	50ml	200ml	RT
Buffer N	20ml	100ml	400ml	RT
Buffer FW	40ml	200ml	800ml	RT
Buffer FE	15ml	75ml	300ml	RT
RNase A (2.3 units/µl)	50µl	250µl	1ml	2-8 °C
Hand Book	1	1	1	–
Certificate of Quality	1	1	1	–

Note:

1. 20µl of RNase A (100mg/ml or 7 units/µl) or 50µl of RNase A from 2.3 units/µl is required for 50 spin columns.

9. Specifications

DNA Purity	1.8-2.0
pDNA Yield	Upto 25µg
pDNA Size	Upto 25Kb
Elution Volume	50µl
Temperature	15-30°C

10. Principle

The **mdi** FastLyse Miniprep system allows us to obtain highly pure pDNA which involves the following:

1. Lysis and neutralization of bacterial culture
2. Capturing of pDNA on to spin column
3. Washing
4. Elution of pDNA to get ultrapure concentrated pDNA

Lysis and neutralization of bacterial culture

The modified alkaline lysis method does away with bacterial pellet formation and resuspension steps.

Capturing of pDNA onto Spin Column

In order to facilitate adsorption of pDNA on to the column, suitable conditions of salt concentration and pH are achieved by addition of buffer N.

Washing

Subsequent to pDNA binding to spin column, unwanted components are washed away by buffer 'FW'.

Elution

Salt concentration and pH of elution buffer is important for elution efficiency, elution occurs in basic conditions and at low salt concentration. Maximum elution efficiency is achieved at pH 7.5-8.5. Elution is done with $\geq$ 30µl of buffer 'FE' to provide highly concentrated ultrapure pDNA.

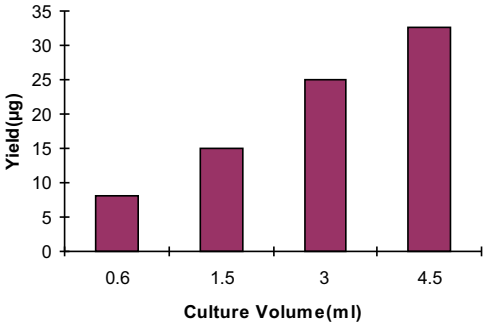
Yield

DNA yield depends on following factors:

1. Culture Volume
2. Elution Volume
3. Point of application of buffer on the column

Host cell	$DH_5\alpha$
Plasmid	pUC
Fragment size	9 Kb
Copy number	500-700
Elution volume	50µl
Culture volume	600µl

Culture Volume Vs. pDNA Yield



11. Important Points to be Considered

1. **Buffer**
   a. All buffers should be stored at room temperature
   b. After adding RNase A to buffer N, store at 2-8°C
   c. For complete neutralization, mix the contents by inverting 2-3 times more
2. **Centrifugation**

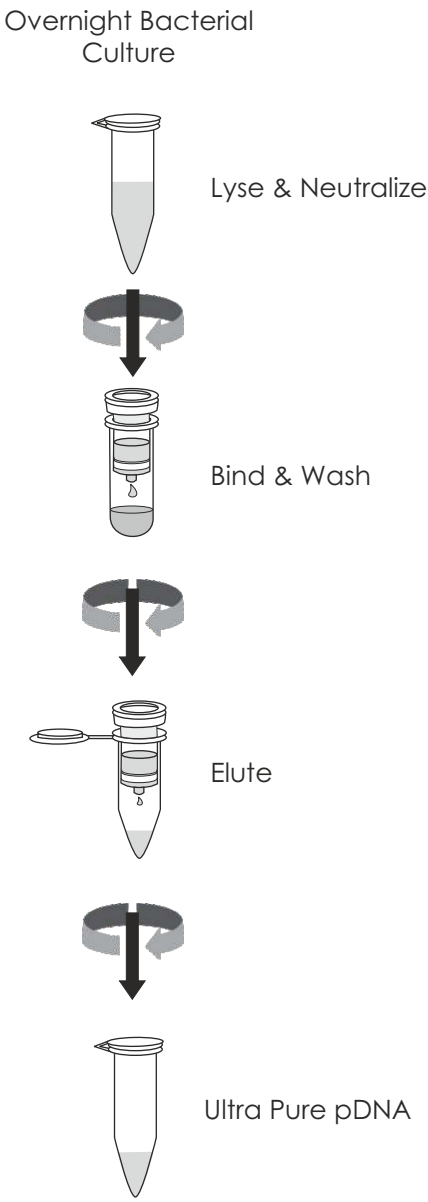
   All centrifugation steps are performed at 10,000 rpm in conventional table top micro centrifuge.
3. **Lysis**

   Avoid excessive lysis, as it can lead to denatured DNA and grow culture under optimal conditions in LB medium.
4. **Washing**

   To avoid contamination of spin column avoid tilting the collection tube after last wash step to ensure that column tip does not contact the flow through.
5. **Elution**

   For maximum elution efficiency, dispense the elution buffer on to the center of column membrane.

12. mdi FastLyse pDNA Miniprep Procedure



13. Protocol

Important Notes

a. Add provided RNase A in the buffer N before use by pipetting 1ml buffer N in to the RNase A vial, mix it, and then transfer the mixture into the buffer N bottle. Store at 2-8°C.

b. Pre cool the buffer N to 2-8°C.

Buffer N may form crystals during cooling, avoid crystals while adding Buffer N to the lysate after buffer L.

c. In case any precipitation is observed in buffer L, re-dissolve it by incubation at 30-37°C for 30 minutes and mix by inversion.

Procedure

1. Add 600µl of bacterial culture grown in LB medium to a 1.5ml micro centrifuge tube.

   Optional: The mdi FastLyse Miniprep kit may also be used with the classical centrifuge based procedure for processing up to 3ml of bacterial culture. The procedure should be modified as follows:

   a) Centrifuge 1.5ml of bacterial culture for 30 seconds at maximum speed.

   b) Discard the supernatant.

   c) Repeat both steps as needed.

   d) Add 600µl of TE buffer (10mM Tris.Cl+ 1mM EDTA, pH 8.0) to the bacterial cell pellet and resuspend completely. Then proceed directly to step- 2.

2. Add 100µl of buffer L and mix by inverting the tube 4-6 times. Proceed to step 3 within 2 minutes.

   Note: After addition of buffer L the solution should become clear from opaque, indicating complete lysis.

3. Add 350µl of cold buffer N and mix throughly by inverting the tube 4-6 times.

Note: Avoid crystals while adding Buffer N to the lysate.

4. Centrifuge at 12,000 rpm for 4 minutes.

   Note: In case of centrifugation at 10,000 rpm, extend the time to 8 minutes.

5. Place the spin column into collection tube and transfer the supernatant (700µl) into spin column without disturbing pellet.

6. Centrifuge at 10,000 rpm for 15 seconds.

7. Discard the flow through and place the spin column back into same collection tube.

8. Now transfer the remaining supernatant (Approx. 200µl) into spin column without disturbing pellet.

9. Again centrifuge at 10,000 rpm for 15 seconds, discard flowthrough.

10. Add 700µl of buffer FW to spin column. Centrifuge at 10,000 rpm for 1 minute 30 seconds.

11. Transfer the column into a clean 1.5ml micro centrifuge tube and add 50µl of buffer FE directly to center of the column membrane and let it stand for 1 minute at room temperature.

    Note: For higher concentration of pDNA, elute with 30µl, but the total recovery will be reduced.

12. Centrifuge at 10,000 rpm for 1 minute to elute the pDNA.

14. Trouble Shooting Guide

A. Little or no DNA

1. Plasmid did not propagate	Please check that the conditions for optimal culture growth described in step 1 were met.
2. Poor bacterial growth	Inoculate from a freshly streaked plate and incubate in a shaker after inoculation at proper speed.
3. Precipitates in Buffer L	Re-dissolve by warming to 37 °C for 30 minutes.
5. Poor cell lysis	Too many cells harvested from an overgrown culture. Use culture of proper O.D. grown in not more than 16 hours.
6. Improper dispensing of the elution buffer	The elution buffer must be dispensed properly on to the center of the membrane.

B: Low quality DNA

1. Nuclease contamination	Use autoclaved plastic and glass wares.
2. RNA contamination	RNase digestion is insufficient. Check that RNase A is added to buffer N.
3. Genomic DNA in eluate	Avoid excessive vortexing or vigorous mixing.

4. Plasmid degradation	Do not incubate in buffer L for more than 2 minutes.
5. Incomplete drying	Results in solvent in the eluate which is difficult to load in gel, also results in reduced yield and difficulties in digestion. Spin the column for 2-3 minutes extra $\geq$10,000 rpm to completely remove the solvent before adding buffer FE.

15. Product Use Limitations

mdi kits are developed and manufactured for research purpose only. The products are not recommended to be used for human, diagnostics or drug purposes for which these should be cleared by the concerned regulatory bodies in the country of use.

16. Product Warranty and Satisfaction Guarantee

All **mdi** products are guaranteed and are backed by our

a. Technical expertise and experience of over 30 years.

b. Special **mdi** process for consistency and repeatability.

c. Strict quality control and quality assurance regimen.

d. Certificate of quality accompanied with each product.

mdi provides an unconditional guarantee to replace the kit if it does not perform for any reasons other than misuse. However, the user needs to validate the performance of the kit for its specific use.

17. Ordering Information

To order please specify as below:

Type		XX	XX	XX	X	Pack Size	
Type	Code					Pack Size	Code
FLPK	FLPK					50	0050
						250	0250
						1000	1000

Example:

FLPK	XX	XX	XX	X	0250

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